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Electrochemical oxidation of uracil and 5-halouracils at pyrolytic graphite electrode

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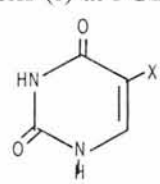
Electrochemical oxidation of uracil and 5-halogenated uracils has been investigated in phosphate buffers of *p*H range 1.2-11.0 using linear and cyclic sweep voltammetry, coulometry, controlled potential electrolysis and spectral studies. A single well-defined 6e, $4H^+$ oxidation peak (I_a) has been observed for uracil whereas halogenated uracils get oxidized in 4e, $3H^+$ step. The presence of halogen group at 5-position of uracil decreases the electron density at the π-bond and the Ep values also shift to less positive potential. The values of the first order rate constant for the disappearance of UV absorbing intermediate have been calculated. The product of electrooxidation has been characterized as alloxan. ECE mechanism has been proposed to account for the formation of alloxan.

Uracil, a pyrimidine base, occurs in ribonucleic acid (RNA) and forms base pair with adenine. Uracil may be utilized preferentially for nucleic acid biosynthesis in tumors[1]. Substitution of hydrogen atom in 5-position of uracil by halogen atoms resulted the drastic changes in the biological properties of uracil[2]. The studies on oxidation-reduction behavior of compounds of biological significance is of considerable value, as they provide deep insight into the biological relevant redox reactions of these compounds. Although actual biological redox systems may be of extreme complexity due to the enzymic interactions, much information can be derived from the study of such compounds in aqueous solutions of known *p*H. The electrochemical reduction of pyrimidines has been the subject of investigation for a long time[3-12] and uracil and its derivatives have been studied at dropping mercury electrode.

However, oxidation studies of pyrimidines have not attracted considerable attention. Hence it was considered interesting to study the electrochemical oxidation of uracil (I) and 5-halouracils at pyrolytic graphite electrode using linear sweep and cyclic voltammetry. A detailed electrooxidation behavior of uracil and 5-halouracils (I) at PGE is presented in this paper.



X = H, F, Cl, Br,I

(I)

Materials and Methods

Uracil and 5-halouracils (Sigma USA) were used as received. Cyclic sweep voltammetric studies were carried out on a Cypress computer controlled electroanalytical system (model CS-1090). All potentials are referred to Ag/AgCl electrode at 22 ±1°C. The number of electrons involved in the oxidation were determined using BAS computer controlled (model MF-9093) system. Controlled potential electrolysis was carried out in H-type cell using pyrolytic graphite plate (6×1.5 cm^2) as a working electrode, cylindrical platinum gauze (1.5×15 cm^2) as a counter electrode and Ag/AgCl as a reference electrode. The electrode surface of pyrolytic graphite electrode was cleaned after recording each voltammogram to remove the adsorbed material by rubbing it with an emery paper. As the surface area of the electrode changed each time due to rubbing, the peak current values observed always showed a variation of ± 10%. Hence an average of at least three runs was taken for the determination of peak current values.

Spectral studies were carried out using Perkin-Elmer Lambda 35 UV/vis. IR spectra of the products were recorded as KBr pellets using a Perkin-Elmer 1600 series FT-IR spectrophotometer. The ^{1}HNMR of the products were recorded in d_6 -DMSO on Bruker AC 300 F instrument.

The stock solutions of uracil and 5-halogenated uracils (1.0 m*M*) were prepared using doubly distilled water. For recording the voltammogram 2.0 mL of the stock solution of the desired compound was mixed

with 2.0 mL of phosphate buffer[13] (ionic strength 1.0 *M*), so that the over all ionic strength of the solution was maintained as 0.5 *M*. A stream of nitrogen gas was passed through the solution for 8-10 min. before recording the voltammograms. In coulometric experiments a potential 100 mV more positive than the oxidation peak potential was applied. The value of (n), the number of electrons involved in oxidation was determined by monitoring exponential decay of current-time curve as reported earlier[14]. The progress of electrolysis of uracil and its derivatives was monitored by recording UV/vis spectra in the range 200-450 nm at different time intervals. For this purpose, 2-3 mL of the electrolyzed solution was transferred each time to 1.0 cm quartz cell and its spectrum was recorded. In a separate experiment, when the absorbance at λ_{max} reduced to ~50%, the circuit was opened and spectral changes of the solution were monitored at different times. Such an experiment provided information about the wavelength region in which UV/vis absorbing intermediate was generated. The change in absorbance with time was monitored at selected wavelengths. The values of rate constant k were calculated from the linear $\log(A-A_\infty)$ versus time plots.

The products of electrooxidation were characterized at *p*H 2.8, 7.2 and 9.1. For the product characterization, 8-10 mg of appropriate uracil was exhaustively electrolyzed at a potential 100 mV more positive than the peak potential of the oxidation peak I_a. Nitrogen gas was continuously bubbled throughout the course of electrolysis. The progress of electrolysis was monitored either by recording cyclic voltammograms or UV spectra at different time intervals. When the oxidation peak disappeared, the electrolysis was stopped and the electrolyzed solution was transferred from the cell and freeze dried. The freeze-dried material was extracted with ethanol (2 × 10 ml) and the ethanolic extract was concentrated. The light pink coloured dried material obtained showed a single spot in TLC (R_f ~ 0.6 in DMSO medium). The material was characterized using melting point, IR and ^{1}HNMR.

Results and Discussion

Voltammetry

Linear sweep voltammetry of uracil and 5-halouracils at a sweep rate of 10 mVs^{-1}, exhibited a single well – defined oxidation peak I_a in the *p*H range 1.2-11.0. The peak potential (Ep) of peak I_a was dependent on *p*H and shifted towards less positive potential with an increase in *p*H. The Ep versus *p*H plots of various uracils were linear. The dependence of Ep on *p*H can be expressed by the following equations: -

Ep (1.2-11.0) = (1839.2-51.81 *p*H) mV. *vs* Ag/AgCl (uracil)
Ep (1.2-11.0) = (1577.5-51.15 *p*H) mV. *vs* Ag/AgCl (5-fluorouracil)
Ep (1.2-11.0) = (1516.3-51.16 *p*H) mV. *vs* Ag/AgCl (5-chlorouracil)
Ep (1.2-11.0) = (1562.0-51.17 *p*H) mV. *vs* Ag/AgCl (5-bromouracil)
Ep (1.2-11.0) = (1481.4-51.01 *p*H) mV. *vs* Ag/AgCl (5-iodouracil)

The linear regression analysis indicated a correlation coefficient of 0.9949, 0.99141, 0.99781, 0.9930 and 0.9955 for uracil, 5-fluoro, 5-chloro, 5-bromo and 5-iodouracils respectively.

Cyclic sweep voltammetry of uracils at a sweep rate of 100 mVs^{-1}, exhibited one well-defined oxidation peak (I_a) when the initial sweep was in the positive direction. In the reverse sweep no peak was noticed. Further reversal of the sweep direction did not exhibit any peak. Some typical cyclic voltammograms of uracil and 5-halouracils are presented in Fig. 1.

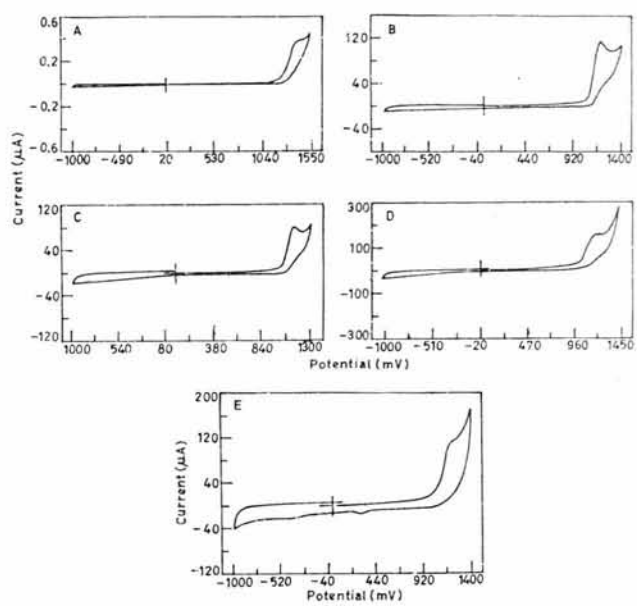


Fig. 1—Cyclic voltammogram of 1m*M* (A) Uracil, (B) 5-fluorouracil, (C) 5-chlorouracil, (D) 5-bromouracil and (E) 5-iodouracil at *p*H 7.2. Sweep rate 100 mVs^{-1}.

The peak current (i_p) for oxidation peak I_a of all the uracils was found to increase with increase in concentration in the range 0.13-1.0 m*M*. The plots of peak current of peak I_a versus concentration of various uracils were linear approximately up to 0.66 m*M* concentration and then showed a tendency to become constant at higher concentrations. Typical i_p versus concentration plot observed for 5-bromouracil is presented in Fig. 2. This behaviour indicated that the electrode reaction associated with peak I_a in these compounds involve adsorption complications[15]. The adsorption of uracil and 5-halouracils at the surface of pyrolytic graphite electrode was further confirmed[16] by the increase in peak current function $i_p/\sqrt{v}$ with increase in log v (Fig. 3).

The effect of sweep rate (v) on peak potential of peak I_a of various uracils was studied at *p*H 7.2 in the sweep range 5-500 mVs^{-1}, however, the peak became broad and merged with the background at sweep rate > 200 mVs^{-1}. The peak potentials of peak I_a for all the compounds were found to shift towards more positive potentials with increase in sweep rate. A typical linear plot observed for 5-fluorouracil is presented in Fig. 4. The plot of Ep versus log v were linear and the dependence of Ep on log v can be expressed by the following equations:

Ep = 13.163 log v + 1456.4 (uracil)
Ep = 13.818 log v + 1103.6 (5-fluorouracil)
Ep = 13.452 log v + 1150.4 (5-chlorouracil)
Ep = 13.863 log v + 1107.9 (5-bromouracil)
Ep = 13.936 log v + 1138.5 (5-iodouracil)

The values of $i_p/\sqrt{v}$ were higher at low sweep rates and the values were lower at high sweep rates. The plot of $i_p/\sqrt{v}$ *vs* v is more or less exponential in nature (Fig. 5) and clearly indicates the nature of the

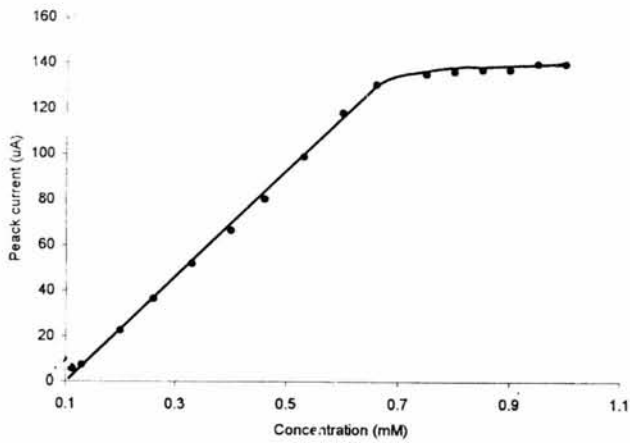


Fig. 2—Dependence of peak current on concentration for peak I_a of 5-bromouracil at PGE, sweep rate 100 mVs^{-1}; *p*H 7.2.

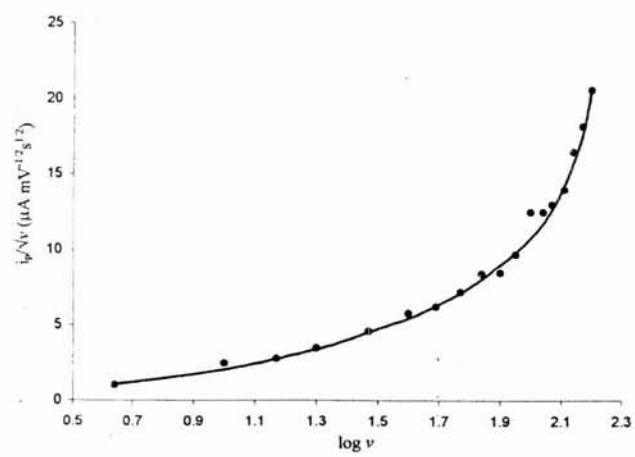


Fig. 3—Variation of peak current function ($i_p/\sqrt{v}$) with the logarithm of the sweep rate for 1m*M* 5-chlorouracil at *p*H 7.2 at PGE.

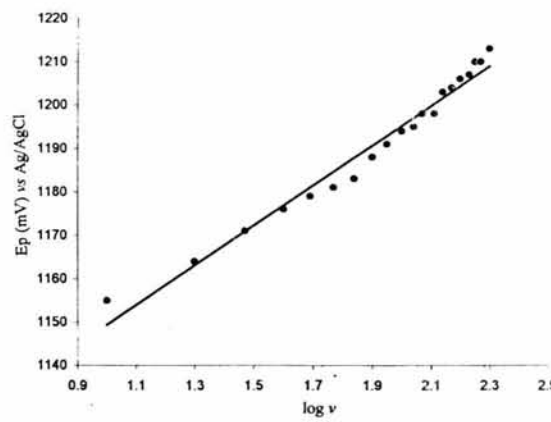


Fig. 4— Plot of peak potential (Ep) *vs* log v for the oxidation peak I_a of 1 m*M* 5-fluorouracil at *p*H 7.2 at PGE.

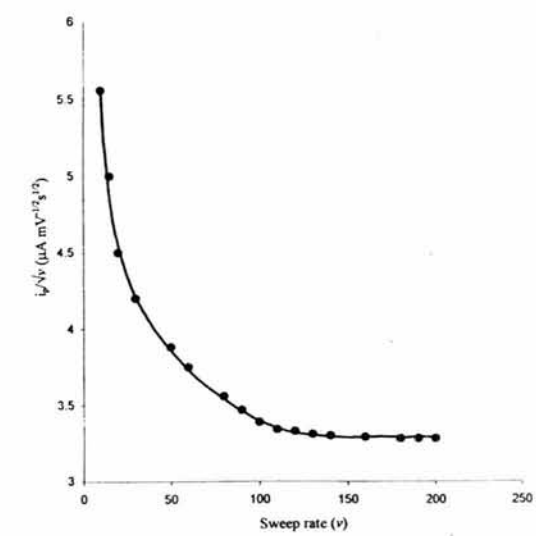


Fig. 5—Variation of ($i_p/\sqrt{v}$) with sweep rate (v) for oxidation peak I_a of 1m*M* uracil at *p*H 7.2 at PGE.

Table 1—Observed λ_{max} of 0.05 m*M* 5-halouracils at different *p*H

Compound	*p*H 2.8		*p*H 7.2		*p*H 9.1	
	λ_{max} 1	λ_{max} 2	λ_{max} 1	λ_{max} 2	λ_{max} 1	λ_{max} 2
Uracil	260.0	194.5	259.1	209.2	260.7	209.2
5-Fluoro	265.5	203.4	283.1	207.5	265.3	207.4
5-Chloro	273.2	208.9	273.3	204.4	288.8	211.1
5-Bromo	276.7	208.9	276.7	210.7	287.5	212.5
5-Iodo	282.1	214.2	283.9	216.0	291.0	217.8

electrode reaction as ECE (electrochemical-chemical-electrochemical) in which chemical reaction takes place between two electron transfer steps[13,17].

The value of (n), number of electrons involved in oxidation of uracil and 5-halouracils were determined at *p*H 2.8, 7.2 and 9.1. It was found that for uracil the value of (n) was 5.6±0.1 in the entire *p*H range whereas the experimental values of n for halouracils were 3.9 ± 0.1.

Spectral studies

UV/vis spectra of uracil and its halo derivatives exhibited two well-defined λ_{max} in the entire *p*H range studied (1.2-11.0). The observed λ_{max} values for various uracils studied at different *p*H are presented in Table 1. The progress of electrolysis of uracils was monitored by recording UV-spectral changes during electrooxidation at different *p*H values to detect the formation of UV-vis absorbing intermediates. Curve 1 in Fig. 6 represents the spectrum of 5-fluorouracil at *p*H 7.2 just before electrooxidation. Upon application of a potential of 1.25 V, corresponding to peak I_a, the absorbance in the wavelength region 250-286 nm initially decreased (curves 2 to 4) while the absorbance in the shorter wavelength region 200-232 nm and 280-450 nm region systematically increased as shown by curves 2 to 8 in Fig. 6. The exhaustively electrolyzed solution (curve 8) recorded after 160 min. of electrolysis, exhibited an absorption maxima ca. 206 nm due to the final product.

At *p*H 2.8, the absorbance increases at both the λ_{max} and the final product also exhibited one well-defined λ_{max} at 206 nm. As the product at both the *p*H exhibited λ_{max} at 206 nm it was concluded that the product formed is the same at both the *p*H. The UV/vis spectral changes observed for uracil and other halogenated uracils were essentially similar to the ones observed for 5-fluorouracil.

In the second set of experiments after electrolyzing the solution of uracils for 40 min. (corresponding to curve 5 in Fig. 6) the potentiostat was open circuit and spectra were recorded at different time intervals. It

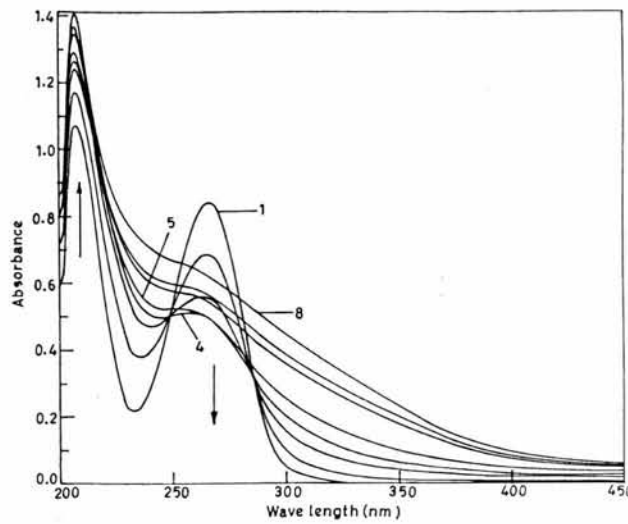


Fig. 6—Spectral changes observed during electrooxidation of 0.05 m*M* of 5-fluorouracil at *p*H 7.2. Pot.1. 25 V *vs.* Ag/AgCl. Curves were recorded at (1) 0; (2) 5; (3) 15; (4) 25; (5) 40; (6) 70; (7) 100; (8) 160 min. of electrolysis.

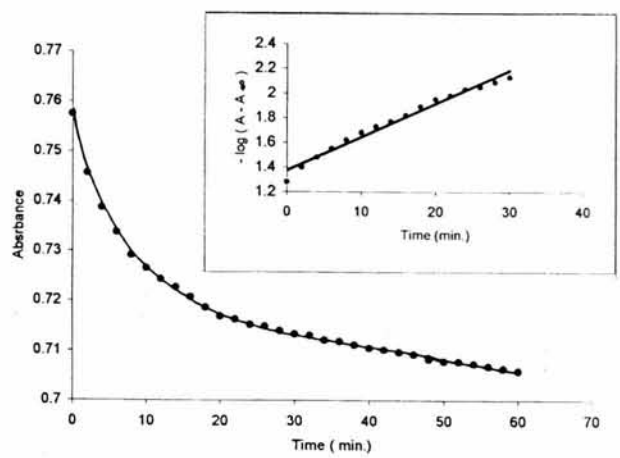


Fig. 7—Observed absorbance *vs* time and log ($A-A_\infty$) *vs* time plots at 210 nm for the first order decay of the UV absorbing intermediate generated during electrooxidation of 5-iodouracil at *p*H 9.1.

was observed that the absorbance in the region 200-300 nm decreased systematically due to the decay of intermediate generated during electrooxidation. Thus, the spectral studies indicated that an intermediate is generated on electrolyzing the solution of uracils,

Table 2—Observed k values for the decay of UV-absorbing intermediate generated during oxidation of 5-halouracils.

Compound	*p*H 2.8		*p*H 7.2		*p*H 9.1	
	λ, nm	$k/10^{-3}s^{-1}$	λ, nm	$k/10^{-3}s^{-1}$	λ, nm	$k/10^{-3}s^{-1}$
Uracil	-	-	230	1.21	210	1.34
	-	-	260	1.23	260	1.36
	-	-	290	1.22	290	1.31
5-Fluoro	210	0.63	210	0.84	210	0.86
	260	0.68	270	0.86	240	0.88
	290	0.60	-	-	270	0.89
5-Chloro	235	0.95	210	1.10	210	1.27
	250	0.97	270	1.20	250	1.23
	275	0.99	310	1.13	320	1.26
5-Bromo	210	0.77	210	1.01	210	1.47
	240	0.68	250	1.10	250	1.42
	270	0.72	270	1.23	280	1.51
5-Iodo	210	0.51	210	0.67	210	1.03
	260	0.56	260	0.59	230	1.19
	280	0.45	280	0.64	290	1.02

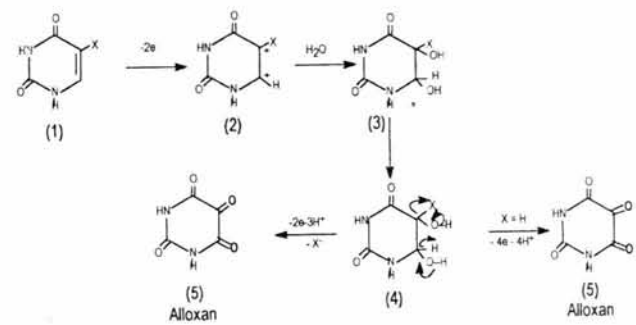
which absorbed in the region 200-300 nm, and decayed due to competitive chemical reactions. The decay of the intermediate was monitored by recording absorbance at selected wavelengths. In each case, the time versus absorbance profile showed an exponential decay. A typical absorbance versus time plot observed for 5-iodouracil at λ_{max} 210 is presented in Fig. 7. The plots of $\log(A-A_\infty)$ versus time (A_∞ and A are final absorbance and the absorbance at the reaction time respectively) were linear. The values of the rate constant k were calculated from the slope value of linear best-fit equation of $\log(A-A_\infty)$ versus time plots at selected wavelengths. Table 2 presents the values of k observed for various uracils at different *p*H. The rate of decay of the intermediate of uracils was dependent on *p*H and the decay was an acid-base catalyzed reaction. It is interesting to note that the rate of decay of the intermediate became almost twice in alkaline *p*H for bromo-and iodouracil, whereas, the rates slightly increase for uracil, fluorouracil and chlorouracil .

Product characterization

The products of the electrooxidation of uracil and 5-halouracils were isolated and characterized at *p*H 2.8 and 7.2. The light pink coloured material obtained on lyophilization of the exhaustively electrolysed solution of halouracils exhibited a single spot in TLC (R_f~0.6) indicating the formation of only one product. The product had a m.p. 240°C. The IR spectrum of the product exhibited strong bands at 3200 (N-H), 1689 (cyclic C=O), 1410 (C-H), 1260, 1170, 1020, 808 and 864 cm^{-1} (C-C and C-N) and was practically superimposable on that of an authentic sample of alloxan. The ^1H NMR spectrum of the product showed one signal at δ = 7.6 (s, 2H) and two weak signals at δ = 3.4 and δ = 2.6 for water molecule, further confirming the product as alloxan monohydrate.

Redox mechanism

The experimental evidences presented above indicate that the electrochemical oxidation of uracil (**I**) proceeds in a 6e, $6H^+$ reaction. The initial electrode reaction involves removal of two π-electrons from the ring to give species (**2**), which is highly unstable and thus readily undergoes hydrolysis to give (**3**). The dihydroxy moiety (**3**) on 4e, $4H^+$ oxidation gives alloxan (**5**) as the final product (Scheme 1). As the oxidation potentials of the two electron transfer steps are very close to each other, a single oxidation peak I_a is noticed. The chemical step is involved in between the two electron transfer steps confirming the ECE nature of the electrode reaction.



Scheme 1—Tentative mechanism proposed for the electrooxidation of uracil and 5-halouracils

A further confirmation to the above mechanism was made in the case of halogenated uracils where the value of (n) was decreased to 4.0. In halogenated uracils, the initial step of oxidation is similar to the unsubstituted uracil in which 2e are removed from the ring. As the species (**2**) is highly unstable it is readily attacked by OH^- to give diol (**3**). Removal of 2e and $3H^+$ then gives alloxan (**5**), however, the attached halogen is also removed as X^-. The presence of X^- in the exhaustively electrolyzed solution of halouracils has also been confirmed by the formation of white precipitate on adding silver nitrate solution. Thus, it is concluded that in the case of halogenated uracils, X^- ions are removed and formation of alloxan takes place as the final product.

Substituents effect

The effect of halogens on the ease of oxidation of uracil was studied qualitatively and quantitatively

Table 3—Variation of $E_{p/2}$ with substituent constant (σ) of 5-halouracils at *p*H 9.1. Sweep rate 100 mVs^{-1} at PGE.

Compound	Ep (mV)	$E_{p/2}$ (mV)	σ
Uracil	1349	666	0.0
5-Fluoro	1112	414	0.52
5-Chloro	1070	398	0.47
5-Bromo	1113	460	0.45
5-Iodo	1140	582	0.38

using Hammett relation. Presence of electron-withdrawing substituents (halogens) at 5-position of uracils shift the $E_{p/2}$ towards less positive value in comparison to unsubstituted compound (Table 3). The shift in $E_{p/2}$ was maximum for chloro substituent whereas the shifts for flouro and bromo substituents were almost equal and less than that of chloro group. The iodo group causes least shift in $E_{p/2}$ values and can be explained on the basis of mesomeric effect of halogens[18]. The decreasing order of mesomeric effect in halogens is Cl > F = Br > I.

The effect of substituents was also determined quantitatively using Hammett equations. The various halo-substituted uracils exhibited the dE_p/dpH values in the same range. As the substituents in uracil are directly attached to the site of oxidation, the system can be represented as (Het-X), where X represents substituents. The values of Hammett substituent constants (σ) for the various groups in such systems are well-known. A plot between $E_{p/2}$ versus σ is presented in Fig.8 and the correlation is found to be (0.7999). The iodo and chloro groups show deviation from the regression line due to the direct field effect i.e., the influence of the outer electric field of halogens. The two opposing effects seem to contribute to this effect vis., the intensity of the electric field (i.e. direct polar effect and steric effect) and the atomic volume of the halogen.

The oxidation of double bond C=C in uracils depends on the electron density available at π-bond (Table 3). The introduction of electron-withdrawing substituents at 5-position may exert a pull on the electrons of the π-bond due to inductive effect and therefore the electron density may decrease. However, the mesomeric effect of halogens play an important role and electron density increases at π-bond and hence the oxidation becomes easier. The $E_{p/2}$ of the substituted derivatives shift towards less positive value in comparison to unsubstituted compound. The value of specific reaction constant (ρ) is found ~ 0.4, which in good agreement with the values reported in the literature for similar systems[19].

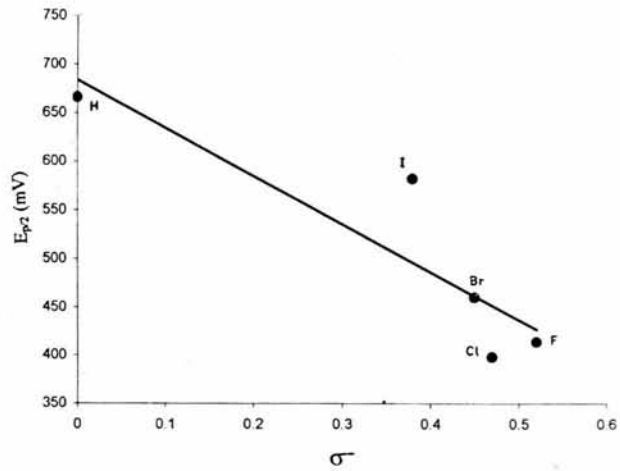


Fig. 8—Variation of $E_{p/2}$ with substituent constant (σ) for 5-halouracils at *p*H 9.1. Sweep rate 100 mVs^{-1} at PGE.

Acknowledgment

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